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Diagnostic approach and effective management of *Malassezia* dermatitis in dogs using itraconazole pulse therapy

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Abstract
Malassezia dermatitis is one of the most common skin diseases in dogs. The present study was taken with the objective of diagnosis and therapeutic management of *Malassezia* dermatitis cases in dogs presented to outpatient medicine unit of Veterinary College Hospital, Bangalore. The suspected animals were screened for clinical signs and confirmed by direct microscopic examination of the clinical samples from affected pets, cultural morphology in Modified Dixon's Agar supplemented with chloramphenicol and cycloheximide and microscopic confirmation of the yeast. Based on the results of direct microscopic examination, the positive cases were treated with itraconazole @5mg/kg b.wt SID, po for two consecutive days in a week for three weeks along with supportive treatment of broad spectrum antibacterial, antifungal shampoo bath twice a week and essential fatty acid supplementation.

Keywords: *Malassezia*, swab smear, culture, Itraconazole pulse therapy

Introduction
Dermatological issues are the single largest group of clinical conditions seen in small animal practice, of which *Malassezia* dermatitis is a relatively common skin disease in dogs which can mimic and also be a secondary complication to a number of other dermatoses. *Malassezia pachydermatis* is a normal inhabitant of healthy canine mucosae and skin. Under the influence of various host predisposing factors, *viz* alteration in host defence mechanism (systemic illness and endocrinopathies) or change in the cutaneous micro climate these commensal yeasts proliferate, turn pathogenic and induce an inflammatory response (Bond *et al*., 2020; Borkar *et al*., 2014) [2, 3]. Enzymes such as keratinase (capable of digesting the keratin protein complex, allowing the organism to burrow deeper into the stratum corneum in the host and elicit an inflammatory reaction), lipase and proteinase produced by *Malassezia* can also contribute to cutaneous inflammation (Brito *et al*., 2009; Carlotti, 2001; Guillot and Gueho, 1995) [4, 5, 8].
Thus, successful case management of the condition is often dependent upon both treating yeast (and any concurrent bacterial) overgrowth with topical or systemic antimicrobial/ antifungal treatments, as well as identifying and correcting the predisposing factors. Itraconazole appears to be effective based on the few clinical trials that have evaluated its efficacy for the treatment of canine *Malassezia* dermatitis. Therapeutic doses recommended are variable with 5 mg/kg once daily (conventional therapy), or for two consecutive days a week (pulse therapy) for three weeks. The pulse therapy approach reflects predicted accumulation of this lipophilic drug in the stratum corneum and reduces costs and likely side-effects of this relatively well-tolerated azole. Upon systemic administration, itraconazole is distributed to the epidermis through passive uptake by keratinocytes in the basal layer at 3–20 folds higher than plasma concentration, and it persists in the stratum corneum for three weeks after the cessation of therapy and thus prevents recurrence of infection. This clearly explains a residual effect of itraconazole over other antifungal therapies (Srivastava and Kothiwala, 2017) [16]. Further, itraconazole has better tissue penetration, has a longer elimination half-life, and is less toxic, compared with ketoconazole. This paper highlights on the diagnosis and therapeutic management of *Malassezia* dermatitis in dogs.

Materials and Methods
Sample collection and processing
A total of ten dogs of different breeds, age and gender that were presented to outpatient unit of

Veterinary College hospital, Bangalore with history and clinical signs suggestive of *Malassezia* such as alopecia, pruritus, greasiness, erythema, hyperpigmentation, hyperkeratinisation, lichenification, rancid odour/ musty/ mal odour formed the study group. Dogs with chronic illness or receiving any systemic antibiotic/anti-fungal medication within the last 30 days were excluded from this study. A detailed history, physical and dermatological examination followed by collection of samples for cytologic examination and yeast culture was obtained from all the ten dogs included in the study. Two Cotton swabs rubbed on the affected sites were used for cytological and cultural studies respectively. Blood (5 ml) was collected for determination of various haematological and biochemical parameters. Number of budding yeasts per field were counted for 15 fields and mean yeast count was recorded pre-treatment and post treatment. As *Malassezia* dermatitis causes moderate to intense pruritus, each dog in the study group was assigned a value from 1-5 for pruritus, where higher number represented more intense pruritus and lower number represented a milder pruritus. (Marsella *et al*., 2000) [9]. Therapeutic evaluation, pre and post treatment was based on cytological evaluation (Mean yeast count) and visual analog scale for pruritus (history) generated on day 0 and day 22. Comparison between pre-treatment (0 day) and post treatment (22 day) was done using paired t-test.

Cytological study

A dry cotton swab was vigorously rubbed on the affected skin surface, on an area that had moist or waxy exudate, and then rolled onto a glass slide. The smear was then air dried and heat fixed. The heat fixed impression smears were stained with Loeffler's alkaline methylene blue for 2-4 minutes. (Selvi *et al*., 2015). As there is no standard accepted number of organisms needed to diagnose *Malassezia* dermatitits, more than one yeast cell per oil immersion field correlated with clinical signs is considered positive for *Malassezia* dermatitis (Pinchbeck *et al*., 2002, Nardoni *et al.*, 2007; Ganguly *et al*., 2013) [13, 10, 7]. Some dogs develop a yeast hypersensitivity, so even if only a few yeasts are found on cytology, treatment may still be indicated. Ten dogs that were positive on direct microscopic/ cytological examination were subjected to treatment with oral itraconazole @ 5mg/kg b wt, sid for two consecutive days in a week for three weeks as recommended by Pinchbeck *et al*., (2002) [13].

Haematology and serum biochemistry

Haematological parameters including Haemoglobin (Hb), Total Leucocyte Count (TLC), Packed Cell volume (PCV), Platelet Count (PLT), were estimated using an automated haematological analyzer (Mindray BC-2800Vet). Biochemical parameters like alanine aminotransferase, total protein, albumin were estimated using semi- automatic serum biochemical analyser (Microlab, India)

Culture

Samples from the sterile swabs were cultured onto Modified Dixon's agar supplemented with chloramphenicol (0.05%) and cycloheximide (0.05%). All the plates were incubated at 37 ^{0}C for about 7 days (Daniel *et al*., 2022; Nunez *et al*., 2022 and Rathnapriya *et al*., 2016) [6, 11, 14] and inspected daily for *Malassezia* growth (Nardoni *et al*., 2007) [10].

Results and Discussions

Dogs presented with dermatological problems suggestive of *Malassezia* dermatitis were screened and ten dogs which were found positive for *Malassezia* by direct microscopy formed the study group. Predominant clinical signs observed in the affected dogs are depicted in table -1. These findings were in accordance with Bond *et al*. (2020) [2] who reported that pruritus, erythema, hyperpigmentation, malodour and traumatic alopecia are the major clinical signs in dogs with *Malassezia* dermatitis (fig 1-5). Hematological and serum biochemical values revealed no statistical significance between pre-treatment (0 day) and post treatment (22 day) group (Table 2). The impression smear stained using methylene blue produced a monochrome image of *Malassezia* organisms which microscopically appeared as small, oval to peanut or footprint shaped (Fig 6).

Out of 10 clinical samples eight isolates suggestive of *Malassezia* spp were obtained on Modified Dixon's agar. The colonies obtained were smooth, round, convex, friable and cream in color (fig 7). Microscopic examination of colonies revealed dark blue coloured footprint shaped organisms on staining with methylene blue for one minute (Bhaswanth *et al*., 2019) [1] (fig 8). Clinical response to itraconazole pulse therapy (fig 9 & 10) was monitored on day 22 after 3 weeks of treatment in terms of cytological evaluation of mean yeast count and pruritus score. The response to therapy on day 22 as compared to day 0 (prior to treatment) was statistically highly significant and there was considerable reduction in the mean yeast count and pruritus score post treatment (Table 3). Thus, an excellent clinical response was noticed in all the ten dogs treated with itraconazole pulse therapy with no side effects recorded (Pinchbeck *et al*., 2002) [13].

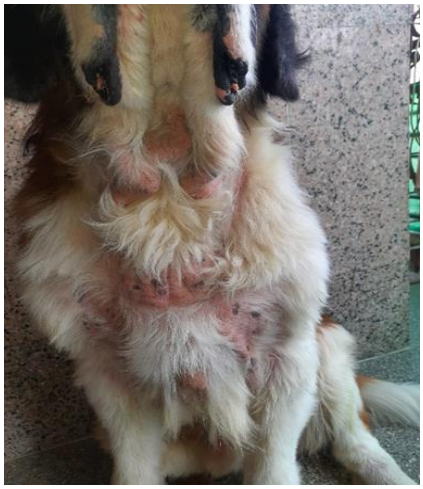

Fig 1: Erythema in a dog with *Malassezia* dermatitis

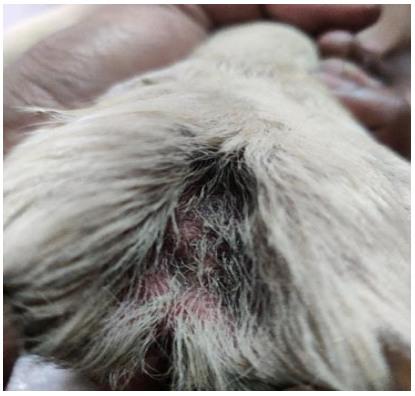

Fig 2: Interdigital erythema and hyperpigmentation in a dog with *Malassezia* dermatitis

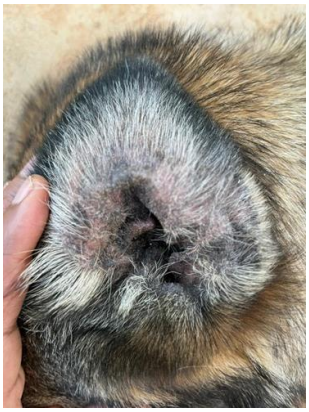

Fig 3: Otitis in a dog with *Malassezia* dermatitis

Fig 4: Latensification and hyperkeratinisation in a dog with *Malassezia* dermatitis

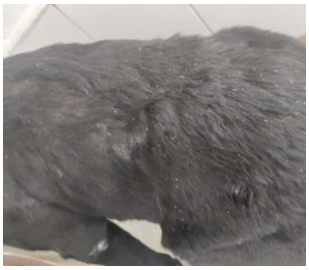

Fig 5: Excessive Scales in a dog with *Malassezia* dermatitis

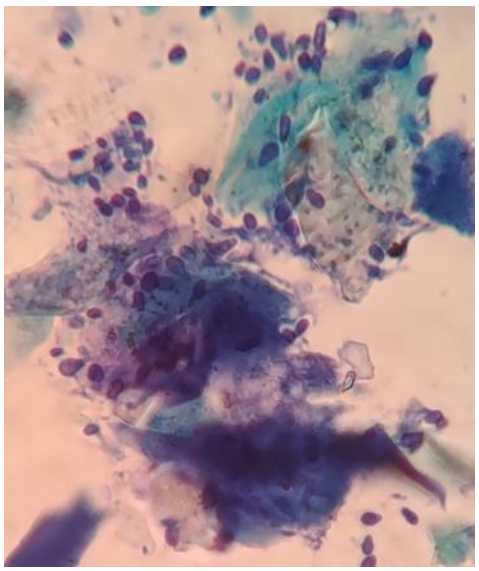

Fig 6: Impression Cytology from a dog revealing different shapes of yeast

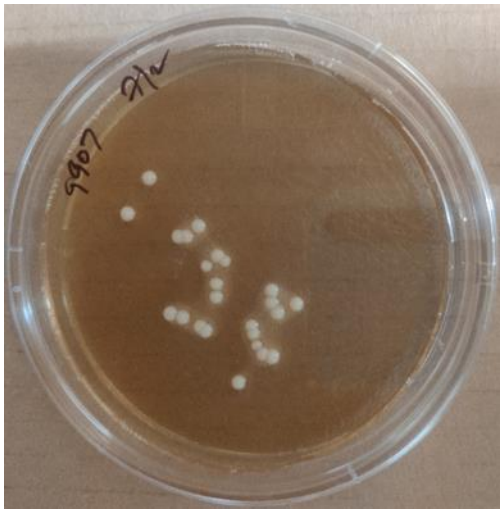

Fig 7: Macroscopic view of culture revealing colonies using modified dixon's agar

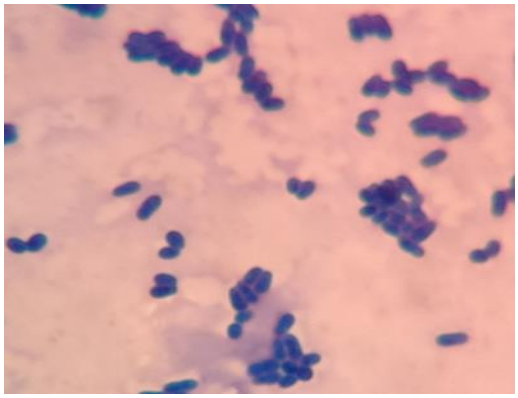

Fig 8: Microscopic view of Stained colonies

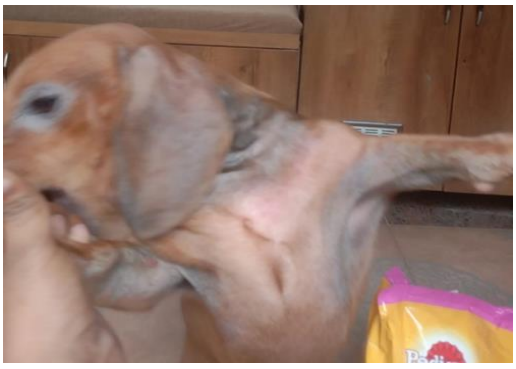

Fig 9: A dog with clinical signs of *Malassezia* dermatitis prior to therapy (0 day)

Fig 10: A dog with clinical signs of *Malassezia* dermatitis post therapy (22 day)

Table 1: Clinical signs observed in dogs affected with *Malassezia* dermatitis

Sl No	Clinical signs	No of animals (n = 10)	Percentage
1.	Pruritus	10	100
2.	Musty/ rancid odour	9	90
3.	Erythema	8	80
4.	Scales	7	70
5.	Hyperpigmentation	5	50
6.	Hyperkeratinisation	4	40
7.	Lichenification	4	40
8.	Epidermal collarettes	6	60
9.	Greasiness	3	30
10.	Otitis	5	50

Table 2: Comparison of hematological and serum biochemical values, pre and post treatment in *Malassezia* affected dogs

Sl. No	Parameter	Mean ± SE		t value	p value
		Pre-treatment 0 day	Post-treatment 22 day		
1	Hb (g/dL)	13.75 ± 0.69	13.22 ± 0.53	1.039	0.3257^{ns}
2	PCV%	41.48 ± 2.07	41.41 ± 1.68	1.714	0.1206 ^{ns}
3	TLC (x 10^3/ cmm)	15.16 ± 2.29	15.44 ± 2.35	0.8617	0.1793 ^{ns}
4	Platelet (($x10^3$/µL)	361.6 ± 53.02	307.5 ± 32.76	1.714	0.1206 ^{ns}
5	ALT(U/L)	27.53 ± 4.121	25.76 ± 2.43	0.5668	0.5847 ^{ns}
6	Total protein (g/dl)	7.91 ± 0.208	7.77 ± 0.32	0.8473	0.4188 ^{ns}
7	Albumin (g/dl)	2.534 ± 0.08	2.660 ± 0.102	0.7025	0.5001 ^{ns}

P ≤ 0.05 = ns (not significant)

Table 3: Comparison of Visual Analog Scale (VAS) for pruritus and yeast load pre and post treatment in *Malassezia* affected dogs

Sl. No	Parameter	Mean ± SE		t value	p value
		Pre-treatment 0 day	Post treatment 22 day		
	Visual analog scale for pruritus	3.300 ± 0.3000	0.9000 ±0.1000	10.85	< 0.0001**
	Yeast load (mean yeast organism per oil immersion field)	4.100 ± 0.6574	1.000 ± 0.2108	5.894	0.0002**

** Significant at 0.01 level (P<0.01)

Conclusion

The present study revealed that *Malassezia* dermatitis can be diagnosed in dogs using a combination of clinical signs, cytology and cultural examination. It was also observed that cytological evaluation and Visual Analog Score for pruritus can be used effectively for assessing therapeutic response. (Patterson and Frank, 2002) [12]. An excellent clinical response, minimal side effects, quick recovery and ease of administration thereby aiding in better owner compliance can be considered as strong valid reasons to suggest oral itraconazole pulse therapy for the effective management of canine *Malassezia* dermatitis.

Conflict of interest: Authors have no conflict of interest in this study.

Acknowledgement

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